Refining High-Throughput *In Vitro-In Vivo* Extrapolation Modeling through Incorporation of Intestinal Toxicokinetics

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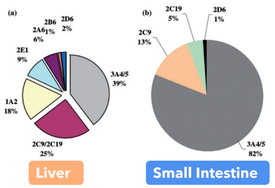
Background

The Toxic Substances Control Act (TSCA) authorizes the U.S. Environmental Protection Agency (EPA) to regulate commercially available substances that do not fall under the jurisdiction of other federal regulations. As of February 2022, there were **42,039** chemicals listed on the TSCA active inventory. Given this number, there is a clear need for a high-throughput (HT) risk-based prioritization and assessment.

HT screening (HTS) for toxicity and toxicokinetic (TK) data are often used with *in vitro-in vivo* extrapolation (IVIVE) modeling to allow the conversion of *in vitro* points of departure (POD) and steady state blood concentration (C_{ss}) values to an administered equivalent dose (AED) in mg/kg/day.

This represents the chemical quantity in mg/kg/day required to achieve an *in vitro* concentration that can be used as an estimate for *in vivo* POD.

Distribution of the major cytochromes P450 (CYPs)

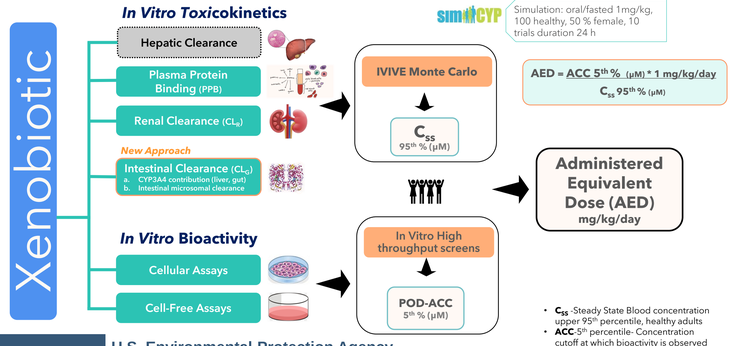


The current HT IVIVE modeling system exhibits a gap between predicted AED levels and actual *in vivo* low effect levels (i.e., PODs) observed in animal studies. This is partially because the HT IVIVE model only considers plasma protein binding (PPB) and hepatic clearance TK data, thus oversimplifying the whole-body metabolism contribution to chemical clearance. Comparing predicted AEDs to *in vivo*-derived PODs, the model proved to be on average 100-fold more conservative.

This study attempts to narrow this gap by incorporating extrahepatic clearance data, namely intestinal clearance, through consideration of CYP3A4 enzymatic contribution to potentially improve the HT IVIVE model prediction capability.

https://www.epa.gov/tsca-inventory/how-access-tsca-inventory
Gunder-Remy U *et al., Drug Metabolism Review (2014) DOI:10.3109/03602532.2014.900565*

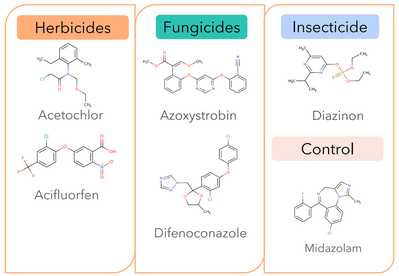
In Vitro In Vivo Extrapolation (IVIVE)



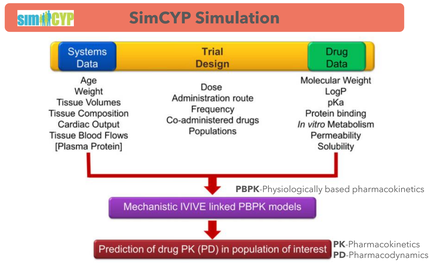
U.S. Environmental Protection Agency
Office of Research and Development

Wetmore B. A., Toxicology (2015) *DOI:10.1016/j.tox.2014.05.012*

Tested Compounds and Modeling



Targeted LC-MS/MS or GC-MS/MS methods were developed for the following compounds to be tested for PPB and Liver/intestinal microsome clearance. 6 more compounds in progress.



SimCYP simulation to acquire representative C_{ss} in a diverse population.

https://comptox.epa.gov/dashboard/
https://www.certara.com/software/simcyp-pbpk/
Jamei M., *Curr Pharmacol Rep* (2016) DOI 10.1007/s40495-016-0059-9

Toxicokinetic *In Vitro* Studies and Modeling

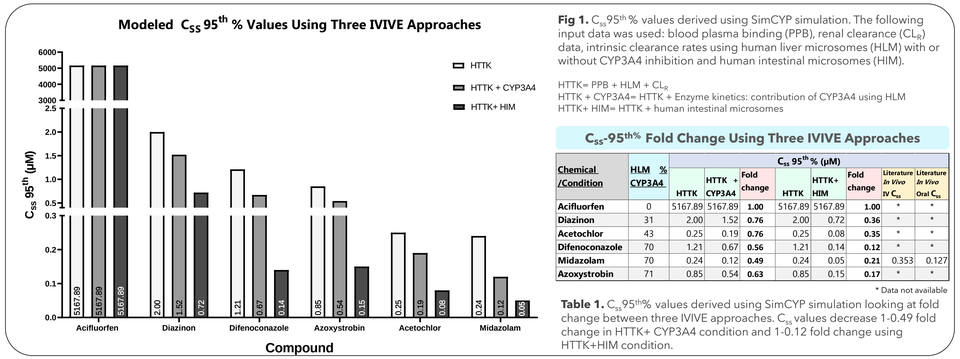


Fig 1. C_{ss} 95th % values derived using SimCYP simulation. The following input data was used: blood plasma binding (PPB), renal clearance (CL_R) data, intrinsic clearance rates using human liver microsomes (HLM) with or without CYP3A4 inhibition and human intestinal microsomes (HIM).

HTTK= PPB + HLM + CL_R
HTTK + CYP3A4= HTTK + Enzyme kinetics: contribution of CYP3A4 using HLM
HTTK+ HIM= HTTK + human intestinal microsomes

C_{ss}-95th% Fold Change Using Three IVIVE Approaches

Chemical /Condition	HLM % CYP3A4	C_{ss} 95th % (μM) HTTK	HTTK + CYP3A4	Fold change	HTTK+ HIM	Fold change	Literature *In Vivo* IV C_{ss}	Literature *In Vivo* Oral C_{ss}
Acifluorfen	0	5167.89	5167.89	**1.00**	5167.89	**1.00**	*	*
Diazinon	31	2.00	1.52	**0.76**	2.00	**0.36**	*	*
Acetochlor	43	0.25	0.19	**0.76**	0.25	**0.35**	*	*
Difenoconazole	70	1.21	0.67	**0.56**	1.21	**0.12**	*	*
Midazolam	70	0.24	0.12	**0.49**	0.24	**0.21**	0.353	0.127
Azoxystrobin	71	0.85	0.54	**0.63**	0.85	**0.17**	*	*

* Data not available

Table 1. C_{ss} 95th% values derived using SimCYP simulation looking at fold change between three IVIVE approaches. C_{ss} values decrease 1-0.49 fold change in HTTK+ CYP3A4 condition and 1-0.12 fold change using HTTK+HIM condition.

SimCYP Derived TK Comparing Three IVIVE Approaches

Parameters	Symbol	HLM * % CYP3A4			HLM 31 % CYP3A4			HLM 43 % CYP3A4			HLM 70 % CYP3A4			HLM 70 % CYP3A4			HLM 71 % CYP3A4		
		Acifluorfen			Diazinon			Acetochlor			Difenoconazole			Midazolam			Azoxystrobin		
		HTTK	HTTK + CYP3A4	HTTK+ HIM	HTTK	HTTK + CYP3A4	HTTK+ HIM	HTTK	HTTK + CYP3A4	HTTK+ HIM	HTTK	HTTK + CYP3A4	HTTK+ HIM	HTTK	HTTK + CYP3A4	HTTK+ HIM	HTTK	HTTK + CYP3A4	HTTK+ HIM
Steady state blood concetration	**Css-95th** (μM)	5167.89	5167.89	5167.89	2.00	1.52	0.72	0.25	0.19	0.08	1.21	0.67	0.14	0.24	0.12	0.05	0.85	0.54	0.15
Clearance	**CL** (L/h)	0.01	0.01	0.01	22.99	22.44	22.99	112.67	110.49	112.67	25.83	25.19	25.83	72.77	72.54	72.77	36.48	35.84	36.48
Oral plasma clearance	**CLpo** (L/h)	0.01	0.01	0.01	45.92	58.79	194.10	416.01	512.19	1839.89	56.82	147.70	972.59	354.10	1060.75	3174.69	79.78	140.83	890.90
Fraction escaping hepatic elimination	**Fh** (Sub)	1.00	1.00	1.00	0.86	0.85	0.86	0.64	0.62	0.64	0.82	0.83	0.83	0.55	0.55	0.55	0.82	0.82	0.82
Fraction escaping gut metabolism	**Fg** (Sub)	1.00	1.00	1.00	1.00	0.9	0.49	1.00	0.90	0.48	1.00	0.67	0.17	1.00	0.62	0.29	1.00	0.74	0.24
Fraction of substrate absorbed from the gut	**fa** (Sub)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Bioavailability of the substrate	**F** (Sub)	1.00	1.00	1.00	0.83	0.69	0.34	0.60	0.49	0.23	0.80	0.46	0.11	0.51	0.26	0.11	0.80	0.53	0.16

* Data not available

Table 2. SimCYP simulation parameters results for three IVIVE approaches for data from **Fig1**.

Smith MT et al., Eur J Clin Pharmacol (1981) DOI 10.1007/s40495-016-0059-9

Administered Equivalent Dose and Bioactivity

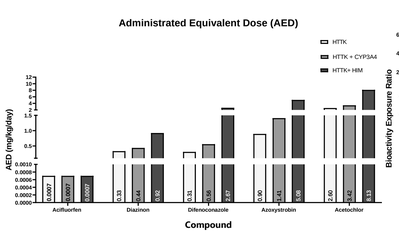


Fig 2. AED evaluation based on C_{ss} 95th % using SimCYP simulation and POD-ACC 5th % for three IVIVE approaches.

POD-ACC 5th % values from CompTox Chemicals Dashboard
https://comptox.epa.gov/dashboard/chemical/invitrodb/

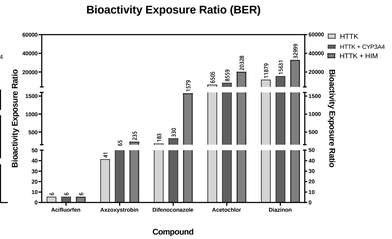
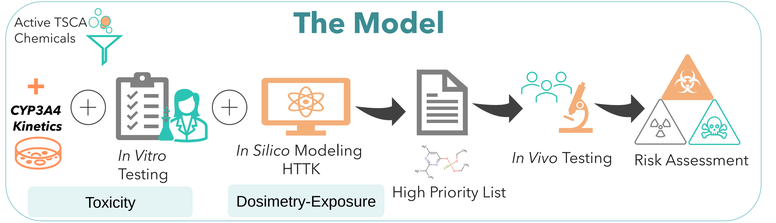


Fig 3. Bioactivity exposure ratio, based on SimCYP simulations and exposure estimates taken from RED document-Registration eligibility documents-EPA.

BER= AED/Exposure Estimate



Summary and Future Work

- This study presents an **IVIVE** approach that incorporates intestinal clearance into estimates of C_{ss} concentration through the consideration of **CYP3A4** contribution and *in vitro* human intestinal microsome clearance rates.
- Adding CYP3A4 enzyme kinetics to the HTTK approach decreases steady state concentration values. In the highly CYP3A4 biotransformed compounds (i.e., over 70 % CYP3A4 contribution) like Difenoconazole, Azoxystrobin and Midazolam, we saw a decrease of 37-50 % in the C_{ss} 95th % values.
- SimCYP simulation using HTTK-HIM data for the highly CYP3A4 biotransformed compounds, resulted in as much as 79-88 % decrease in C_{ss} 95th % values compared to default HTTK.
- When comparing our C_{ss} 95th % modeled Midazolam values to human *in vivo* data, we saw an agreement between our HTTK-CYP3A4 C_{ss} 95th % value and the *in vivo* oral administered dose C_{ss} (Smith et al., 1981).
- Calculations of AED and BERs demonstrate the differences that intestinal clearance consideration could make in risk-based prioritization assessment.

Future Work

- Complete data generation across all 12 compounds
- Incorporate *in vitro* intestinal absorption data and evaluate impact on the IVIVE approach
- Evaluate factors contributing to the differences between CYP3A4 and HIM IVIVE approach